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A Model for Primitive Neuroectodermal Tumors in Transgenic Neural Transplants Harboring the SV40 Large T Antigen

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Using retrovirus-mediated transfer of the SV40 virus large T antigen into neural transplants, we have observed a high incidence of primitive neuroectodermal tumors (PNET). These neoplasms developed in 8 of 14 (57%) neural grafts after latency periods of 176 to 311 days. Histopathologically, the tumors exhibited features of human PNET such as formation of neuroblastic rosettes and immunocytochemical evidence for neuronal differentiation, synaptogenesis, and focal astrocytic differentiation. All neoplasms showed a striking migratory potential. The presence of the large T gene in the tumors was demonstrated by polymerase chain reaction-mediated amplification of a specific 242 bp segment of large T and DNA sequence analysis. Large T antigen was identified in tissue sections using an immunocytochemical reaction with the monoclonal antibody Pab 108. Cell lines were established from several tumors and subjected to G418 selection. Secondary tumors induced by intracerebral transplantation of these cells retained the characteristic morphological and immunocytochemical properties of PNETs. These experiments demonstrate a considerable transforming potential of SV40 large T antigen for neural precursor cells. The long latency period suggests that neoplastic transformation initiated by the large T gene requires additional spontaneous mutations of cooperating cellular genes. Because the mechanism of transformation by large T antigen appears to involve complex formation with and inactivation of cellular tumor suppressor gene products, these cell lines may serve as an interesting tool to search for novel neural tumor suppressor genes. (Am J Pathol 1994, 144:556–564)

The concept of primitive neuroectodermal tumors (PNET) has recently received considerable attention from neuropathologists.[1,2] These neoplasms have been extensively characterized through histopathological and immunohistochemical studies and evidence has been provided for a histogenesis of PNET from a neural precursor cell with the potential for both neuronal and glial differentiation.[3,4] Attempts to identify the cell of origin and the molecular pathogenesis of PNETs have, however, not succeeded. An animal model for these intriguing neoplasms would be able to address these issues.

We have recently demonstrated that retrovirus-mediated gene transfer into neural transplants can be successfully used to study *in vivo* effects of oncogenes on the brain.[5–9] The rationale is to introduce and express foreign genes in fetal rat brain transplants using as genetic vehicle a replication-defective retroviral vector that harbors the gene of interest. Because these transplants will develop into highly differentiated central nervous system (CNS) tissue, they provide an excellent model for the developing rat brain. Retroviral vectors can efficiently be introduced and expressed in all major neuroectodermal and mesenchymal cell types of the developing and adult CNS, including neurons, astrocytes, neural precursor, and vascular endothelial cells. Tissue-specific regulatory sequences are not required. In contrast to transgenic animals, stable integration and expression of retrovirally transmitted genes will only succeed in a small fraction of the fetal donor cells.

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This approach has the advantage that, in analogy to the natural conditions of tumor evolution, integration and expression of a transforming gene occur only in a limited number of donor cells that develop within the normal environment of the graft.

Using this experimental approach, we have introduced SV40 large T antigen into fetal brain transplants to study potential effects of this transforming gene on the developing rat CNS. Large T antigen is the transforming gene of simian virus 40, a monkey papova virus with tumorigenic activity in various animal species.[10] There is recent evidence that SV40 or a closely related virus that differs from human polyomaviruses JC and BK may have an etiological role in the development of ependymomas and choroid plexus papillomas in childhood.[11] It has been shown in transgenic mice that expression of SV40 large T antigen can induce choroid plexus papillomas, retinoblastomas, and tumors of the pineal gland.[12–14] An intriguing property of this gene is the transformation mechanism used by large T antigen. It encodes a nuclear phosphoprotein with specific DNA binding activity and a striking potential for complex formation with cellular proteins some of which have been identified as the products of cellular tumor suppressor genes.[15] These properties have made large T antigen an interesting candidate for gene transfer experiments into CNS transplants. We herein present data indicating that tumors induced by retrovirally expressed large T antigen in neural grafts represent a potent new model for human PNET.

Materials and Methods

Packaging Cell Lines and Retroviral Vectors

The retroviral vector used for these experiments encodes both the SV40 early region and a neomycin phosphotransferase resistance gene (NEO). A splice mechanism generates transcripts encoding SV40 large T only, without production of small T.[16] A schematic map of the retroviral construct is shown in Figure 1. By conferring resistance to the cytotoxic antibiotic G418, the NEO gene greatly facilitates the selection of packaging cells that produce high titers of the replication-defective retroviral vector. After retroviral infection, it allows for selection of target cells that stably integrate and express the retroviral construct. Packaging cells were kept in 10% Iscove's modified Dulbecco's medium containing 10% fetal calf serum and antibiotics. Retroviral supernatants were collected overnight at >80% confluency then sterile filtered (Millipore; 0.22 μM) and concentrated by centrifugation at 12,000 *g* for 2 hours and frozen. The titers used for infection of fetal donor cells were in the order of 10^6 focus forming units per ml. No helper virus was detected in the vector preparations.

Preparation and Infection of Fetal CNS Donor Cells

Fisher rats (F344, obtained from Charles River, Hannover, Germany) were mated overnight; the morning on which sperms were detected in the vaginal smear was defined as E0.5. At day E14.5 and E13.5 of gestation, pregnant animals were anesthetized, the embryos removed, and the entire fetal brain dissected using a stereomicroscope. Special care was taken to avoid contamination with leptomeninges and choroid plexus. The pooled fetal brains obtained from one litter were then enzymatically dissociated (0.25% trypsin, 0.1% DNase in phosphate-buffered saline, 10 minutes at room temperature) and gently triturated to obtain a single-cell suspension. Subsequently, the cells were incubated with supernatant of the vector producer cell line in the presence of polybrene (3 μg/ml) for 4 hours at 37 C and 5% CO_2. Before stereotaxic injection, the cells were washed with Hank's balanced salt solution to remove free retroviral particles.

Stereotaxic Transplantation

Adult F344 rats (150 to 200 g body weight) were used as recipients. Animals were anesthetized, placed in a stereotaxic frame (Narishige, Tokyo, Japan), and received a stereotacic injection of 10 μl cell suspension containing 10^6 cells into the center of the left caudoputamen. Postoperatively, host animals were closely monitored for signs of neurological impairment.

Microscopic and Immunohistochemical Analysis of the Grafts

Animals with severe neurological symptoms, eg, retardation or epileptic seizures, were killed. The brain was removed and in cases with a macroscopic tumor, one fragment of the tumor tissue was used for cell culture and DNA extraction. The remainder was fixed in 4% paraformaldehyde (dissolved in 100 mM phosphate buffer, pH 7.4) then cut in frontal sections and embedded in paraffin. The 4-μM sections were stained with a combination of H&E and luxol fast blue.

Antibodies directed to distinct cell types in the graft included polyclonal rabbit antisera to glial fibrillary acidic protein (GFAP; Dakopatts, Copenhagen, Denmark), neuron-specific enolase (NSE; Dakopatts), synaptophysin (a generous gift from Dr. R. Jahn, Martinsried), and monoclonal antibodies to SV40 large T antigen (a generous gift from Dr. W. Deppert, Heinrich-Pette-Institut, Hamburg, Germany), synaptophysin (SY-38, Boehringer Mannheim, Mannheim, Germany), neurofilament protein (Beckton Dickinson), and vimentin (Dakopatts). As a control monoclonal antibody of the same IgG1 isotype, the monoclonal antibody Lu-5 to human panepithelial antigen was used. All reactions with this control antibody were consistently negative. To avoid problems with rat/mouse cross-reactivity of immunological reagents, affinity-purified secondary antibodies were used. Polyclonal antibodies were detected with a commercial avidin-biotin-peroxidase kit (Dakopatts) and monoclonal antibodies with a peroxidase antiperoxidase reaction.[5]

Generation of Cell Lines

Four grafts harboring neoplasms (Table 1, tumors 2, 3, 8, and 9) were dissected from the host brain then mechanically dissociated and plated in tissue culture flasks with Iscove's modified Dulbecco's medium containing 10% fetal calf serum and antibiotics. As soon as significant outgrowth of cells became detectable, G418 (Geneticin, GIBCO; 0.4 mg/ml) was added to the medium.

Polymerase Chain Reaction (PCR) Analysis and Direct Sequencing of PCR Products

Specific primers were deduced from the nucleotide sequence of SV40 large T antigen.[10] The primers were as follows: 5′-GGAGTGGAAAGAGAGATTGG and 5′-AGGACTGAGGGGCCTGAAAT. PCR was performed with 500 ng of genomic DNA, 10 pmol of each primer, 200 μM concentrations of deoxynucleotide triphosphates, 10 mM Tris (pH 8.8), 50 mM KCl, 1 mM $MgCl_2$, and 2.5 units of Taq polymerase (Cetus Corporation) in a total volume of 100 μl. Thirty-five cycles of denaturation (95 C) for 70 seconds, annealing (53 C) for 70 seconds, and extension (72 C) for 120 seconds were conducted. After amplification, 10 μl were analyzed on a 0.8% agarose gel and stained with ethidium bromide. For PCR-based DNA sequencing, 70 μl of the PCR reaction were electrophoresed on a 0.6% agarose gel. The amplified bands were excised from the gel and the DNA was recovered by electroelution and ethanol precipitation; dried DNA was dissolved in 12 μl of distilled water. Sanger dideoxynucleotide sequencing was performed using ^{35}SdATP and primers for amplification.[17,18] The template-primer mixture (4 μl and 10 pmol primer) was heated at 95 C for 5 minutes and immediately placed in liquid nitrogen. An aliquot containing 20 mM 10% dimethylsulfoxide and 5 μCi ^{35}SdATP was added to each of the four termination mixtures and incubated at 37 C for 10 minutes with 2 units Sequenase version 2.0 (USB). Samples were mixed with 4 μl stop solution, heated at 80 C for 2 minutes, and immediately loaded onto a 6% polyacrylamide-7 M urea gel. Gels were fixed with 10% acetic acid and 10% methanol then dried and autoradiographed for 5 days.

Results

Gene Transfer into Fetal Brain Transplants and Tumor Development

In two independent experiments, a total number of fourteen rats received neural transplants of E14.5 (12 recipients) and E13.5 (2 recipients) fetal brain cells infected with the MoMuLV-SV40 large T retroviral vector. The construct encodes a neomycin resistance gene and the SV40 early region under the control of the 5′ retroviral LTR (Figure 1). All animals with severe neurological symptoms, ie, epileptic seizures, hemiparesis, or physical retardation, and all animals that survived without evidence for neurological impairment for periods exceeding 1 year were killed and subjected to a detailed neuropathological examination. One animal died at 240 days after stereotaxic transplantation. Of 14 recipient animals, eight (57%) developed large, solid tumors originating from the graft within 5 to 11 months after transplantation. The tumor incidence was identical in both experiments. The mean interval for clinical tumor development was 249 days (range 176 to 311 days). One transplant showed a microscopic tumor at day 176 after transplantation. The results are summarized in Table 1.

Histopathological Characterization of the Tumors

Postmortem examination revealed the presence of large solid tumors at the transplantation site. A representative example is shown in Figure 2. On histopathological analysis, these neoplasms were strikingly uniform in morphology. All tumors exhibited

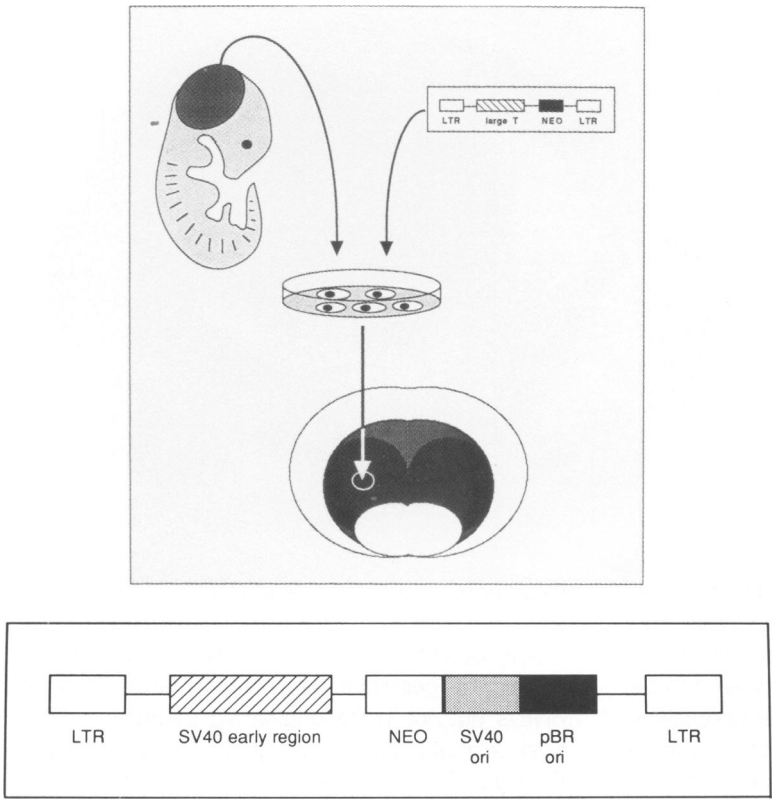

Figure 1. *Experimental approach for retrovirus-mediated transfer of SV40 large T into CNS transplants.* **A:** *Primary cell suspensions of E14.5 fetal rat brains are infected with the retroviral vector encoding large T and stereotaxically transplanted into the caudoputamen of adult recipient animals.* **B:** *The replication-defective retroviral vector is derived from Moloney murine leukemia virus. Its genome contains the SV40 early region that gives rise to large T antigen and the* Escherichia coli *neomycin phosphoribosyltransferase resistance gene (neo) for* in vitro *selection of recombinant cells.*

Table 1. *Tumor Development in Neural Transplants Harboring SV40 Large T*

Series no. 1			Series no. 2		
Animal No.	Latency (days)	Phenotype	Animal No.	Latency (days)	Phenotype
1	176	Micro. tumor	8	164	PNET
2	176	PNET	9	238	PNET
3	240	PNET	10	256	PNET
4	301	PNET	11	307	PNET
5	311	PNET	12	341	No tumor
6	389	No tumor	13	341	No tumor
7	389	No tumor	14	341	No tumor
	Mean latency period: 257 days			Mean latency period: 241 days	

Series 1 and 2 represent independent transplantation experiments with fetal donor cells exposed to the retroviral vector encoding SV40 large T. The tumor incidence was 57% (4 of 7 host animals each) in both series. A microscopic neoplastic focus was found in the transplant of animal no. 1.

features of PNET and were indistinguishable from human PNETs (Figure 3A). Round cells with hyperchromatic nuclei and sparse cytoplasm represented the main cell type. These tumors showed a potential for both neuronal and glial differentiation as demonstrated by the formation of neuroblastic rosettes, immunohistochemical detection of NSE and synaptophysin, and focal expression of GFAP in neoplastic cells (Figure 3B and C).

With a polyclonal antiserum to synaptophysin, immunoreactivity was present in all eight tumors. Immunohistochemical reactions with an antiserum to GFAP showed clusters of immunoreactive tumor cells in five of the eight PNETs (Table 2). Neurofilament protein was not detectable on paraffin sections of the tumor cells. Synapse formation within the tumor could also be demonstrated by electron microscopy (data not shown). One of the tumors contained a prominent population of giant cells with expression of synaptophysin. These elements may represent aberrantly differentiating neoplastic neurons derived from the tumor. A distinct tumor component with the histopathological appearance of a gemistocytic astrocytoma was associated with the PNET in one animal. Seven of the eight PNETs exhibited a striking migratory potential (Figure 3D).

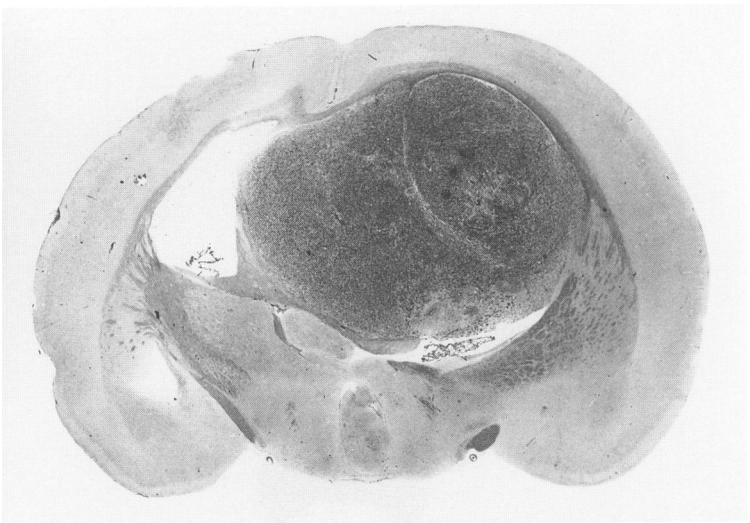

Figure 2. *Frontal section of a host brain harboring a large solid tumor that has largely replaced the neural graft (H&E stain).*

This resulted in a diffuse neoplastic infiltration into the contralateral hemisphere and infratentorial compartments of the host brain in several animals. In some instances, the migratory path appeared to be guided by myelinated fiber tracts. Other neoplasms were not observed in these grafts and there was no evidence for developmental alterations in the residual transplants that showed the characteristic microscopic appearance of organotypic neural grafts.

Retransplantation of Tumor-Derived Cell Lines

Four primary PNETs were explanted *in vitro* and permanent cell lines were successfully established from all four tumors. To eliminate contaminating reactive astrocytes and to exclude neoplastic cells without expression of the retrovirally transduced NEO and large T genes, these cell lines were selected for neomycin resistance using the drug G418. Characteristic properties of these cell lines were the formation of cell clusters and extension of prominent neuritelike processes (Figure 4). These features indicate a potential for neuronal differentiation *in vitro* and further support a histogenesis of the original tumors from neural progenitor cells. Secondary tumors induced by intracerebral transplantation of these four cell lines retained the characteristic morphological and immunocytochemical properties of PNETs (Figure 3F).

Detection of the Large T Gene and of Large T Antigen in the Tumors

The presence of a large T genome in the tumors was demonstrated by PCR-mediated amplification of a segment of large T and DNA sequence analysis. The expected PCR product of 242 bp was readily detectable in all tumor specimens examined and in the cell lines derived from large T-induced PNETs but not in the unaffected cerebellum of the host animals. Figure 5A shows a representative PCR gel. The nature of the PCR products was verified by PCR-based DNA sequencing analysis that revealed the characteristic sequence of SV40 large T (Figure 5B). We have also been able to identify the large T protein in the tumors using a monoclonal antibody to SV40 large T antigen. Immunohistochemical reactions showed an intense nuclear immunoreactivity characteristic of large T in virtually all tumor cells. Endothelia within the tumor tissue and the cells of the adjacent host brain were consistently negative. A representative example is depicted in Figure 3E.

Discussion

The tumors induced by retrovirus-mediated transfer of the large T gene into neural grafts exhibit several features that strongly indicate that these neoplasms represent an intriguing model for PNETs. Histopathologically, they are virtually indistinguishable from human PNETs.[1,19] A prominent blue cell population and the formation of neuroblastic rosettes are among the morphological properties characteristically encountered in these experimental tumors and in their human counterparts.[3,4] The bimodal differentiation capacity with immunohistochemical evidence for both focal astrocytic and more widespread neuronal differentiation is also typically seen in human PNETs.[20,22] From these striking parallels

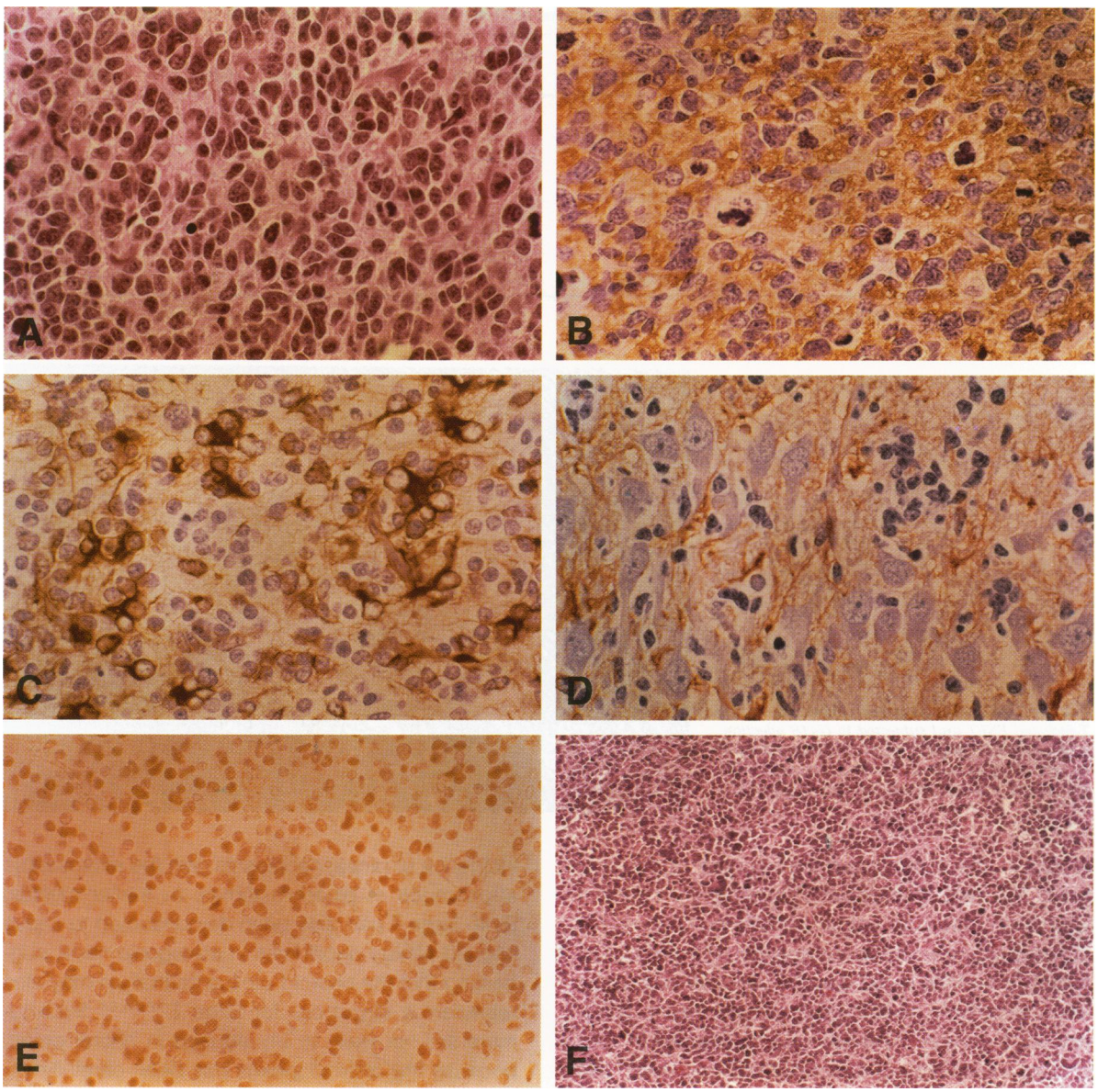

Figure 3. *Histopathological characterization of the tumors induced by retroviral expression of large T in CNS transplants.* A: *Characteristic morphology of a large T-induced PNET. Note the formation of neuroblastic (Homer-Wright) rosettes indicating an early stage of neuronal differentiation (H&E stain).* B: *Neuronal differentiation of the tumor cells demonstrated by strong immunoreactivity with a polyclonal antibody to the synaptic vesicle protein, synaptophysin.* C: *Astrocytic differentiation of the tumor. The reaction with an antiserum to GFAP reveals clusters of immunoreactive neoplastic cells.* D: *Extensive infiltration of tumor cells into the hippocampus of the adjacent host brain. Immunohistochemical reaction with a polyclonal antibody to synaptophysin.* E: *Immunohistochemical detection of SV40 large T antigen in the tumor tissue. The monoclonal antibody Pab 108 to large T was used for the reaction. Note the characteristic nuclear staining pattern and the absence of immunoreactivity in capillary endothelial cells.* F: *Histopathological appearance of a secondary transplant obtained after intracerebral injection of a tumor-derived cell line. The PNET morphology is completely preserved.*

in morphological appearance and differentiation pathways, we would conclude that both neoplasms are likely to be derived from the same progenitor cell of the brain. However, the nature of this cell of origin is still elusive. Cell lines derived from large T-induced PNETs may be amenable to studies on the histogenesis and the regulation of differentiation mechanisms in these pluripotent cells.

Migration and infiltration into the adjacent brain pose a major therapeutic challenge for the management of PNETs and other malignant neural tumors in humans. The prominent migratory behavior of neoplastic cells in large T-induced PNETs may also provide a model system to approach this problem. In several cases, the migration potential was so pronounced that clusters of tumor cells could be de-

Table 2. *Histopathological and Immunohistochemical Features of SV40 Large T-Induced Neoplasms in Neural Grafts*

Animal No.	Diagnosis	Infiltration into Host Brain	Neuroblastic Rosettes	NSE	SYN	GFAP
2	PNET	Extensive	+	+	+	+
3	PNET	Extensive	–	+	+	–
	Astrocytoma	Moderate	–	–	–	+
4	PNET	Extensive	+	+	+	+
5	PNET	Extensive	+	+	+	+
8	PNET	Extensive	–	+	+	+
9	PNET	Moderate	+	+	+	–
10	PNET	Extensive	+	+	+	–
11	PNET	Extensive	+	+	+	+

SYN, immunoreactivity for synaptophysin.

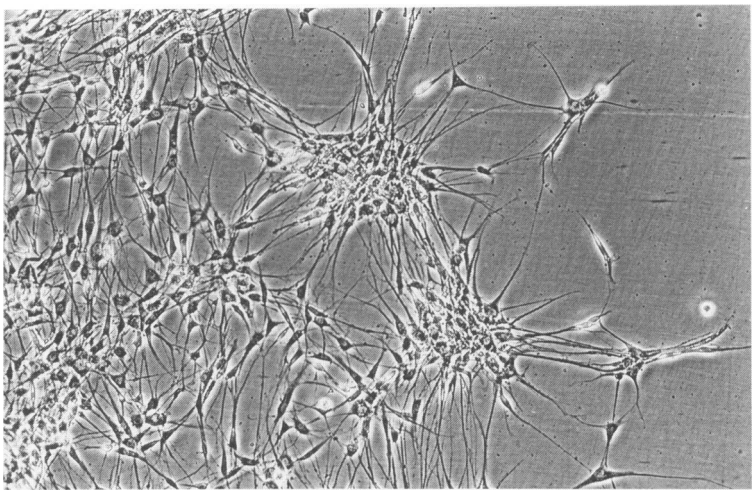

Figure 4. *Cell line derived from a large T-induced PNET and subjected to G418 selection. A characteristic property of this cell line is the formation of cell clusters with long cell processes. The cells exhibit cytological features of primary neuronal cells.*

tected in the contralateral hemisphere of the host brain. The frequent association of tumor cell infiltrates with myelinated fiber tracts and with the cerebral microvasculature would be compatible with a role of interacting surface molecules on neuronal processes and capillaries in mediating tumor cell invasion. A significant migratory behavior was maintained in secondary transplants of tumor-derived cell lines. This may allow combined *in vitro-in vivo* studies.

With the exception in one animal of a single tumor component exhibiting histopathological features of an astrocytoma, all neoplasms observed in this study had the characteristic morphology of primitive neuroectodermal tumors. This indicates that precursor cells of the developing CNS are the principal target for large T-induced transformation in neural grafts. Other neural and nonneuroectodermal cell types, some of which have also been shown to express the retroviral construct, appear to tolerate this gene without tumor development or other morphologically detectable alterations. A similar observation was made by Theuring et al[14,23] in transgenic mice harboring the SV40 large T cDNA under the control of a Moloney murine sarcoma virus enhancer element. These animals developed a high incidence of pineal neoplasms with the microscopic appearance of pineoblastomas. In earlier studies, SV40 large T had also been demonstrated to cause choroid plexus papillomas in transgenic mice, suggesting that the choroid plexus epithelium may also represent a target cell for large T.[12,13,24] Such tumors are not likely to be induced in neural grafts, because the choroid plexus has been carefully excised from the fetal donor tissue.

Latency periods between 164 and 311 days were observed for the development of macroscopic tumors after stereotaxic transplantation of large T-exposed neural donor cells. This suggests that the process of neoplastic transformation initiated by large T antigen requires the contribution of spontaneously mutated cellular growth-controlling genes that cooperate with large T in inducing PNETs. The nature of such complementing cellular genes is still unknown. However, cell lines established from these tumors will provide a substrate to search for such genes. Potential candidates include members of the *myc* gene family that occasionally exhibit genomic amplification in human primitive neuroectodermal tumors.[25]

SV40 large T uses an intriguing mechanism of transformation. It involves the interaction with and

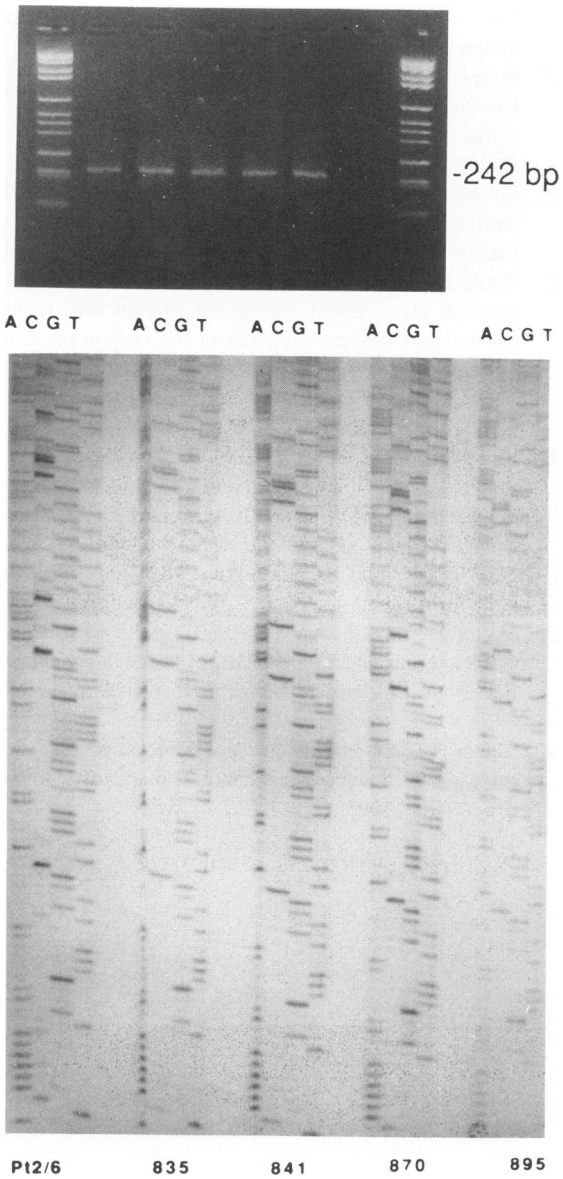

Figure 5. *Identification of the SV40 large T gene in the tumors.* **A:** *PCR-mediated amplification of a 242 bp fragment of the large T sequence. Note the 242 bp product present in all tumors and in the positive control cell line (***lane 2***).* **Lanes 1** *and* **8,** *DNA marker;* **lane 2,** *cerebellar cell line harboring SV40 large T (positive control);* **lanes 3** *to* **6,** *four primary tumors induced by retrovirus-mediated transfer of large T into neural grafts;* **lane 7,** *normal rat cerebellum (negative control).* **B:** *PCR-based DNA sequencing of the fragment shown in* **A**. *All tumor samples show the identical sequence specific for SV40 large T.*

inactivation of cellular regulatory proteins.[15] Several of these proteins have been identified as the products of cellular tumor suppressor genes including the RB-1 and p53 genes. Recent studies have demonstrated that RB-1 and p53 are not usually affected in human PNETs including medulloblastomas indicating that other not yet identified cellular transforming genes are involved in the pathogenesis of PNET.[17,25] Due to its capacity for complex formation with host nuclear proteins, the large T antigen incorporated in the tumors and tumor cell lines described in this report may constitute a potent marker to detect the products of such genes. An initial strategy to identify these molecules will concentrate on immunoprecipitation analysis of protein-protein complexes between large T antigen and cellular polypeptides.

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References

1. Kleihues P, Burger PC, Scheithauer BW: Histological typing of tumors of the nervous system. Berlin, Springer, 1993
2. Rorke LB: Classification and grading of childhood brain tumors, overview and statement of the problem. Cancer 1985, 56:1848–1849
3. Aguzzi A, Wiestler OD, Kleihues P: Differentiation in the medulloblastoma: an immunohistochemical evaluation of 247 cases from the therapy trial SIOP/GPO MED 84. Verh Dtsch Ges Pathol 1988, 72:284–287
4. Rorke LB: The cerebellar medulloblastoma and its relationship to primitive neuroectodermal tumors. Neuropathol Exp Neurol 1983, 42:1–15
5. Aguzzi A, Kleihues K, Heckl K, Wiestler OD: Cell type specific tumor induction in neural transplants by retrovirus mediated oncogene-transfer. Oncogene 1991, 6:697–708
6. Brüstle O, Aguzzi A, Talarico D, Kleihues P, Wiestler OD: Angiogenic activity of the K-*fgf/hst* oncogene in neural transplants. Oncogene 1992, 7:1177–1183
7. Wiestler OD, Brüstle O, Eibl RH, Radner H, Aguzzi A, Kleihues P: Retrovirus-mediated oncogene transfer into neural transplants. Brain Pathol 1992, 2:47–59
8. Wiestler OD, Brüstle O, Eibl RH, Radner H, von Deimling A, Plate K, Aguzzi A, Kleihues P: A new approach to study the molecular basis of neoplastic transformation in the brain. Neuropathol Appl Neurobiol 1992, 18: 443–453
9. Wiestler OD, Aguzzi A, Schneemann M, Eibl RH, von Deimling A, Kleihues P: Oncogene complementation in fetal brain transplants. Cancer Res 1992, 52:3760–3767
10. Tooze J: DNA Tumor Viruses. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1980

11. Bergsagel DJ, Finegold MJ, Butel S, Kupsky WJ, Garcea RL: DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. N Engl J Med 1992, 326:988–993
12. Brinster RL, Chen HY, Messing A, van Dyke T, Levine AJ, Palmiter RD: Transgenic mice harbouring SV40 T-antigen genes develop characteristic brain tumors. Cell 1984, 37:367–379
13. Messing A, Pinkert CA, Palmiter RD, Brinster RL: Developmental study of SV40 large T antigen in transgenic mice with choroid plexus neoplasia. Oncogene Res 1988, 3:87–97
14. Theuring F, Götz W, Balling R, Korf HW, Schulze F, Herken R, Gruss P: Tumorigenesis and eye abnormalities in transgenic mice expressing MSV-SV40 large T-antigen. Oncogene 1990, 5:225–232
15. Green MR: When the products of oncogenes and anti-oncogenes meet. Cell 1989, 56:1–3
16. Jat PS, Cepko CL, Milligan RC, Sharp PA: Recombinant retroviruses encoding simian virus large T antigen and polyomavirus large and middle T antigens. Mol Cell Biol 1986, 6:1204–1217
17. Ohgaki H, Eibl RH, Wiestler OD, Yasargil M, Newcomb E, Kleihues P: p53-mutations in nonastrocytic human brain tumors. Cancer Res 1991, 51:6202–6205
18. Sanger F, Nicklen S, Coulsen A: DNA sequencing with chain terminating inhibitor. Proc Natl Acad Sci USA 1977, 74:5363–54–67
19. Hart NM, Earle KM: Primitive neuroectodermal tumours in children. Cancer 1973, 32:172–188
20. Burger PC, Grahmann FC, Bliestle A, Kleihues P: Differentiation in the medulloblastoma: a histological and immunohistochemical study. Acta Neuropathol 1987, 73:115–123
21. Schwechheimer K, Wiedemann B, Franke WW: Synaptophysin: a reliable broad-range marker for medulloblastomas. Virchows Arch Pathol Anat 1987, 411:53–59
22. Gould VE, Jansson DS, Molenaar WM, Rorke LB, Trojanowski JQ, Lee VM, Packer RJ, Franke WW: Primitive neuroectodermal tumours of the central nervous system: patterns of expression of neuroendocrine markers, and all classes of intermediate filament proteins. Lab Invest 1990, 62:498–509
23. Korf H-W, Götz W, Herken R, Theuring F, Gruss P, Schachenmayr W: S-antigen and rod-opsin immunoreactions in midline brain neoplasms of transgenic mice: similarities to pineal cell tumors and certain medulloblastomas in man. J Neuropathol Exp Neurol 1990, 49: 424–437
24. Marks JR, Lin J, Hinds P, Miller D, Levine AJ: Cellular gene expression in papillomas of the choroid plexus from transgenic mice that express the simian virus 40 large T antigen. J Virol 1989, 63:790–797
25. Bigner SH, Vogelstein B: Cytogenetics and molecular genetics of malignant gliomas and medulloblastoma. Brain Pathol 1990, 1:12–18